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Lack of Anti-GOR Antibody Among Subjects With GB Virus C/Hepatitis G Virus RNA

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Homologies were sought between the putative amino acid sequences of GB virus C/hepatitis G virus (GBV-C/HGV) and the GOR epitope or the liver/kidney microsome-1 (LKM-1) epitope, which share partial sequence identity with the hepatitis C virus (HCV) polyprotein. Anti-GOR antibody (anti-GOR) was assayed among 100 subjects with GBV-C/HGV RNA. Twenty-one and 25 subjects were coinfected with hepatitis B virus (HBV) or HCV, respectively. Homologies were found between the NS5 or E2 polyproteins of GBV-C/HGV and the GOR epitope or the LKM-1 epitope, respectively. These segments of GBV-C/HGV polyproteins sharing identity with the GOR or the LKM-1 epitope were well conserved among three genotypes of GBV-C/HGV. However, only 1 of 55 subjects (1.8%) with GBV-C/HGV RNA, but not with HBV or HCV, was positive for anti-GOR. The positivity for anti-GOR among the group with GBV-C/HGV RNA alone was significantly lower than that among the groups with HCV RNA ($P < 0.01$ and $P < 0.05$, respectively). Only 2 of 55 subjects (3.6%) with GBV-C/HGV RNA alone exhibited elevation of alanine aminotransferase. The incidence of liver dysfunction among the group with GBV-C/HGV RNA alone was significantly lower than the incidence among the groups with GBV-C/HGV RNA and hepatitis B surface antigen (HBsAg) or HCV RNA ($P < 0.01$ and $P < 0.01$, respectively). These data indicate that 1) there is no association between GBV-C/HGV infection and the presence of anti-GOR, and 2) GBV-C/HGV infection is not related to chronic liver dysfunction. ***J. Med. Virol. 55:129–133, 1998.*** © 1998 Wiley-Liss, Inc.

KEY WORDS: GOR epitope; anti-GOR; GB virus C/hepatitis G virus; liver/kidney microsome-1 epitope; hepatitis C virus

INTRODUCTION

Anti-GOR antibody (anti-GOR) is an autoantibody induced by hepatitis C virus (HCV) infection and is assumed to target both a core gene product of HCV and a host component because of the homology between the GOR epitope and a viral epitope on the core protein in HCV [Mishiro et al., 1991]. This is thought to be one of reasons why HCV infection may present with autoimmune disease-like features, such as long-lasting inflammation [Mishiro et al., 1991]. Manns et al. [1991] suggested that the partial sequence identity between a portion of a cytochrome P-450 monooxygenase (P450IID6) harboring the liver/kidney microsome-1 (LKM-1) epitope and the HCV polyprotein as well as the GOR epitope could be related to type 2 autoimmune hepatitis (AIH). Although GB virus C/hepatitis G virus (GBV-C/HGV) was reported to be related in its evolution to HCV with a genome organization similar to that of HCV [Simons et al., 1995; Leary et al., 1996], GBV-C/HGV infection does not lead to chronic liver damage,

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TABLE I. Subjects Seropositive for GBV-C/HGV RNA

	No.	Gender (M:F)	Age (years)	Seropositive for HBsAg	Seropositive for HCV RNA	Seropositive for ALT > 30 IU/liter	Country of origin
General population	49	13:36	31.9 ± 12.1	4	4	4	Japan (n = 5) China (n = 2) Russia (n = 21) Uzbekistan (n = 10) Brazil (n = 11)
Subjects with liver diseases	36	28:8	41.2 ± 16.1	16	16	21	Japan (n = 10) China (n = 8) Korea (n = 3) Mongolia (n = 12) Brazil (n = 3)
Subjects with other diseases	15	10:5	43.9 ± 15.0	1	5	6	Japan (n = 5) Korea (n = 10)
Total	100	51:49	37.0 ± 14.8	21	25	31	

GBV-C/HGV: GB virus C/hepatitis G virus; HBsAg: hepatitis B surface antigen; HCV: hepatitis C virus; ALT: alanine aminotransferase.

and GBV-C/HGV differs from HCV, in that it does not encode a core protein [Muerhoff et al., 1996; Alter et al., 1997a,b; Okamoto et al., 1997]. The sequence homology between the GBV-C/HGV polyprotein and either the GOR epitope or the LKM-1 epitope is still unknown. The association between GBV-C/HGV infection and the appearance of anti-GOR or type 2 AIH has not been reported, although Ichijo et al. [1997] reported that GBV-C/HGV does not play a casual role in the development of type 1 AIH. In this study, the homologies were examined between the putative amino acid sequences of three different genotypes of GBV-C/HGV and the GOR epitope or the LKM-1 epitope, and tests were carried out for the presence of anti-GOR in the sera of subjects with GBV-C/HGV RNA.

MATERIALS AND METHODS

Subjects

To identify subjects seropositive for GBV-C/HGV RNA, the presence of serum GBV-C/HGV RNA was examined among the general population, subjects with liver disease, and subjects with other diseases in various geographic areas, including Japan, Korea, China, Mongolia, Uzbekistan, Russia, and Brazil (Table I). GBV-C/HGV RNA was detected in the sera of 100 subjects; then, the presence of serum anti-GOR was examined in these 100 subjects. Forty-nine were from the general population, and 36 and 15 were subjects with liver diseases and other diseases, such as hematological diseases or chronic renal failure, respectively. The subjects consisted of 51 males and 49 females with a mean age of 37.0 years (6–85 years). In addition to being positive for GBV-C/HGV RNA, 21 and 25 of them were also positive for hepatitis B surface antigen (HBsAg) or HCV RNA, respectively. Thirty-one had liver dysfunction, as judged by a serum alanine aminotransferase (ALT) concentration of greater than 30 IU/liter.

Serological Tests

Serum samples were examined for HBsAg by using passive hemagglutination (Fujirebio, Tokyo, Japan). Anti-GOR in serum was detected by enzyme immunoassay (Institute of Immunology, Tokyo, Japan). The specificity of anti-GOR in anti-GOR-positive samples that were negative for HCV or GBV-C/HGV RNA was determined by an absorbance test using the GOR peptide. The ALT concentration in all serum samples was measured.

Detection of HCV RNA

Serum HCV RNA was detected by using the nested reverse transcription-polymerase chain reaction (RT-PCR) with primers from the 5′-untranslated region (5′UTR) of the HCV genome, as reported previously [Okamoto et al., 1992].

Detection of GBV-C/HGV RNA

RNA was extracted from 100 μL of serum by using the Sepa Gene-RVR kit (Sanko, Tokyo, Japan), precipitated with isopropanol, and washed with ethanol. Complementary DNA (cDNA) was synthesized from the RNA at 37°C for 1 hour by using Moloney murine leukemia virus reverse transcriptase (MMLV-RT; GIBCO BRL, Gaithersburg, MD). Serum GBV-C/HGV RNA was detected by using the seminested RT-PCR with primers derived from the 5′UTR of GBV-C/HGV genome, as reported previously [Orito et al., 1996]. First-round PCR was carried out by using 1/20 of the cDNA, the sense primer 5gf2 (5′-GGTTGGTAGGTCGTAAATCCCGGTCA-3′) and the antisense primer 5gr4 (5′-GCGACGTGGACCGTACRTGGGCGT-3′). First-round PCR consisted of 35 cycles of amplification of 94°C for 1 minute, 55°C for 45 seconds, and 72°C for 1 minute. Using 1/50 of the first-round PCR product, the second-round PCR was undertaken under the same conditions as the first-round PCR, using the sense primer 5gf3 (5′-TGGTAGCCACTATAGGTGGGT-3′) and the antisense primer 5gr4. The amplified products were analyzed by electrophoresis on 3% agarose gels, stained with ethidium bromide, and observed under ultraviolet light.

Homology Search

The homology searches between the putative amino acid sequences derived from the full-length GBV-C/

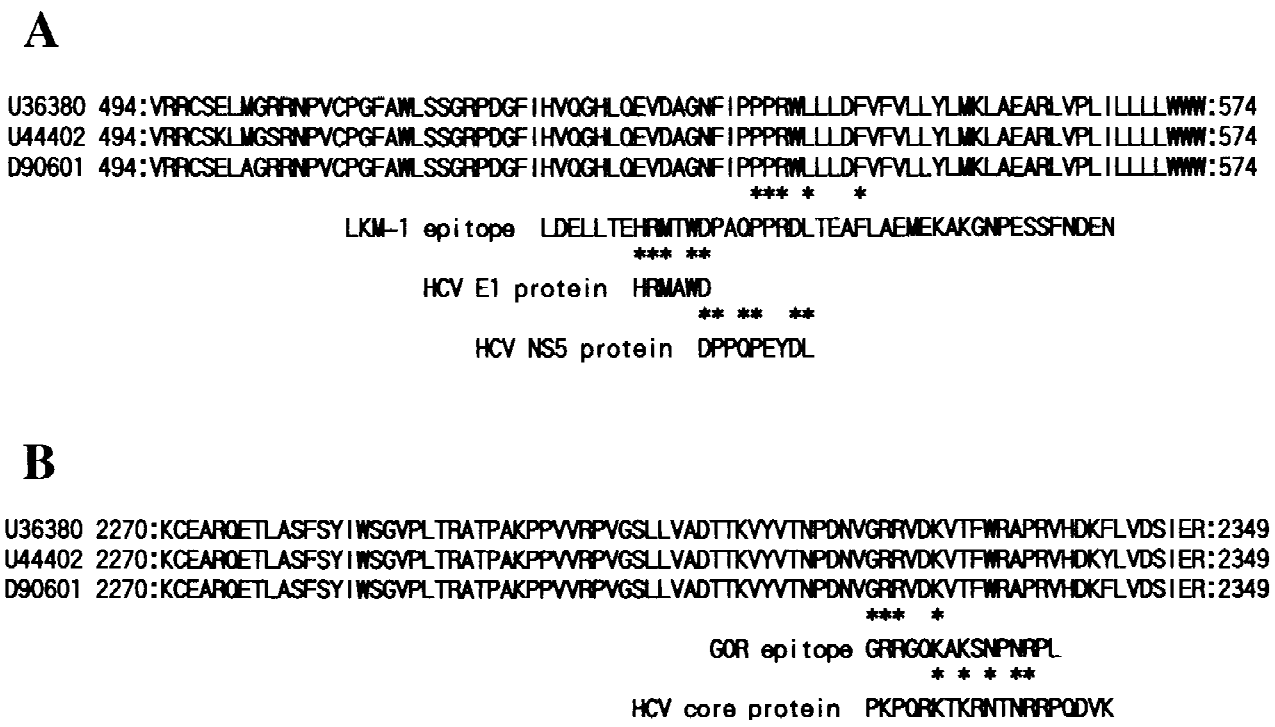

Fig. 1. Homology between the GOR epitope or a portion of the amino acid sequence of P450IID6 harboring the liver/kidney microsome (LKM-1) epitope and the putative GB virus C/hepatitis G virus (GBV-C/HGV) polyproteins of three different genotypes: prototype GBV-C (accession no. U36380) [Simons et al., 1995], HGV (no. U44402) reported by Linnen et al. [1996], and another type (no. D90601) reported by Okamoto et al. [1997]. The amino acid position, as numbered from the methionine predicted as the site of translation initiation by Muerhoff et al. [1996], is indicated. Asterisks denote homologous amino acids between both sequences. **A:** A portion of the E2 region of GBV-C/HGV polyprotein shares homology with the LKM-1 epitope. The homologies reported by Manns et al. [1991] between the LKM-1 epitope and hepatitis C virus (HCV) polyproteins are also indicated. **B:** A portion of the NS5 region of GBV-C/HGV polyprotein shares homology with the GOR epitope. The homology reported by Mishiro et al. [1991] and Hosein et al. [1992] between the GOR epitope and the HCV polyprotein is also shown.

HGV nucleotide sequences and the amino acid sequences of the GOR epitope or the portion of P450IID6 harboring the LKM-1 epitope were carried out by using GENETYX-MAC software (version 8.0; Software Development, Tokyo, Japan).

Statistical Analysis

Fisher's exact test was used for statistical analysis. $P < 0.05$ was considered statistically significant.

RESULTS

Homologies Between GBV-C/HGV Polyprotein and the GOR Epitope or the LKM-1 Epitope

Amino acid sequence homologies were sought between the GOR epitope (GRRGQKAKSNPNRPL) or a portion of the amino acid sequence of P450IID6 harboring the LKM-1 epitope (LDELLTEHRMTWDPAQPPRDLTEAFLAEMKAKGNP) and the putative GBV-C/HGV polyproteins encoded by GBV-C/HGV genomes of three different genotypes: prototype GBV-C [Simons et al., 1995], HGV described by Linnen et al. [1996], and another type reported by Okamoto et al. [1997] (Fig. 1). The E2 region of the GBV-C/HGV polyproteins, according to the cleavage site proposed by Leary et al. [1996], exhibited sequence homology with the LKM-1 epitope. The NS5 region of the polyproteins exhibited homology with the GOR epitope. However, these polyproteins exhibited a lower degree of sequence identity with the GOR epitope or the LKM-1 epitope than did HCV polyproteins [Manns et al., 1991; Mishiro et al., 1991]. These segments of the polyproteins among the three types of GBV-C/HGV, which were sharing sequence identity with the GOR epitope or the LKM-1 epitope, were well conserved.

Positivities for Anti-GOR and Incidence of Liver Dysfunction Among Subjects With GBV-C/HGV RNA

Table II shows the positivities for anti-GOR and the incidence of liver dysfunction among the 100 subjects with GBV-C/HGV RNA. One subject was positive for GBV-C/HGV RNA, HBsAg, and HCV RNA. Twenty subjects were positive for GBV-C/HGV RNA and HBsAg. Twenty-four subjects were positive for GBV-C/HGV RNA and HCV RNA. Fifty-five subjects were positive for GBV-C/HGV RNA alone. The positivities for anti-GOR of the subject with GBV-C/HGV RNA, HBsAg, and HCV RNA; the group with GBV-C/HGV RNA and HBsAg; the group with GBV-C/HGV RNA and HCV RNA; and the group with GBV-C/HGV RNA alone were 1 of 1 subjects (100%), 0 of 20 subjects (0%), 15 of 24 subjects (62.5%), and 1 of 55 subjects (1.8%),

Table II. Serum Anti-GOR and ALT Elevation Among Subjects With GBV-C/HGV RNA

Virus status										
GBV-C/HGV RNA	HBsAg	HCV RNA	Seropositve for anti-GOR					ALT > 30 IU/liter		
+	+	+	1/1	(100%)	*			1/1	(100%)	
+	+	–	0/20	(0.0%)	**	*		12/20	(50%)	
+	–	+	15/24	(62.5%)	**			16/24	(66.7%)	**
+	–	–	1/55	(1.8%)				2/55	(3.6%)	**

*$P < 0.05$.
**$P < 0.01$.

respectively. Only 1 of the 55 subjects (1.8%) with GBV-C/HGV RNA alone was positive for anti-GOR. The positivity for anti-GOR among the group with GBV-C/HGV RNA alone was significantly lower than that among the two groups with HCV RNA ($P < 0.01$ and $P < 0.05$). The positivity for anti-GOR among the group with GBV-C/HGV RNA and HBsAg was also significantly lower than that among the two groups with HCV RNA ($P < 0.01$ and $P < 0.05$). Only 2 of the 55 subjects (3.6%) with GBV-C/HGV RNA alone exhibited serum ALT concentrations higher than 30 IU/liter. The incidence of liver dysfunction among the group with GBV-C/HGV RNA alone was significantly lower than among the groups with GBV-C/HGV RNA and either HBsAg or HCV RNA ($P < 0.01$ and $P < 0.01$, respectively).

DISCUSSION

It was reported that the cross recognition between the GOR epitope and the core protein of HCV may explain why HCV infection exhibits signs and symptoms of autoimmune characters, such as long-lasting inflammation [Mishiro et al., 1991]. Hosein et al. [1992] found a region of weak homology between the core protein of HCV and a 15-amino-acid length of the GOR epitope. They reported that the peptide from HCV could inhibit 50% of the binding of anti-GOR to GOR peptide-coated wells. This phenomenon demonstrates the potential importance of the mimicry in driving an autoimmune response. We found homology between the GOR epitope and a part of the putative NS5 gene product of GBV-C/HGV, although it was weaker than that between the GOR epitope and the product of HCV (Fig. 1). Moreover, these segments of three types of GBV-C/HGV polyproteins sharing homology with the GOR epitope were invariant.

The amino acid sequences of the E1 or NS5 regions of HCV polyprotein and the LKM-1 epitope derived from a host protein exhibit significant homology to each other as well as to the GOR epitope [Manns et al., 1991], although, in a review, Eddleston [1996] showed that the mechanisms underlying the association between HCV infection and AIH with the LKM-1 antibody, type 2 AIH, are not clear. Homology was also found between the LKM-1 epitope and a part of the putative E2 gene product of GBV-C/HGV, although it was also weaker than that between the LKM-1 epitope and the HCV polyproteins (Fig. 1). These segments of three types of GBV-C/HGV polyproteins sharing homology with the LKM-1 epitope were also invariant.

Thus, we predicted that the mimicry between the GOR epitope or the LKM-1 epitope and the conserved regions of GBV-C/HGV might induce autoimmune responses, such as the appearance of anti-GOR in serum. However, only 1 of 55 subjects (1.8%) with GBV-C/HGV RNA alone was positive for anti-GOR. There was no significant difference between the positivity for anti-GOR in these 55 subjects and that of blood donors reported by Mishiro et al. [1990]. The positivity for anti-GOR was high among the groups with HCV RNA but not among the group with GBV-C/HGV RNA alone. Miyakawa et al. [1994] reported that they found no cross-reactive antibody to both the LKM-1 epitope and the HCV polyproteins in their anti-HCV$^+$, type 2 AIH patients, despite considerable homology in several regions between the LKM-1 epitope and the HCV polyproteins. Molecular mimicry may not always lead to cross recognition by antibodies. Moreover, only 2 of 55 subjects (3.6%) with GBV-C/HGV RNA alone exhibited ALT concentrations higher than 30 IU/liter. The incidence of liver dysfunction among the subjects with HCV RNA and/or HBsAg was higher than that among the subjects with GBV-C/HGV RNA alone. These data indicate that 1) there is no association between GBV-C/HGV infection and the presence of anti-GOR in serum, despite the partial identity between the GOR epitope and a part of the putative gene product of GBV-C/HGV, and 2) GBV-C/HGV infection is not related to chronic liver dysfunction.

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